Microflora - microfauna interactions in Antarctic moss peat decomposition processes

D.D. WYNN-WILLIAMS

British Antarctic Survey
Natural Environment Research Council
Madingley Road, Cambridge CB3 0ET (ENGLAND)

Summary :

In two consecutive years at Signy Island, maritime Antarctic, a spring peak of respiratory activity was observed during the decomposition of freshly-sampled intact peat cores from moss communities. The peak was followed by a sharp decline within 1-2 weeks. The composition of the microflora changed concurrently, a decrease in yeast numbers being conspicuous. Although the availability of soluble nutrients is probably the prime factor affecting this decline, predation of microorganisms by invertebrates is thought to be contributory. Simulations *in vitro* of such microbe-microfauna interactions in intact peat cores support this hypothesis.

Key words : interactions, Antarctic peat, simulations, respiration.

I. Introduction

A series of inter-related projects on moss communities at the Signy Island terrestrial reference sites (SIRS) in the maritime Antarctic have demonstrated seasonal fluctuations in population density and composition of the microflora and microfauna (COLLINS *et al.*, 1975; DAVIS, 1981). Changes in total peat respiration in a moss turf were not wholly explained by changes in ground temperature, water, or the availability of energy substrates. During 1975-77, it was observed that microfaunal populations increased during the decline in microbial biomass which followed the spring peak in O_2-uptake at the time of the thaw (CALDWELL, 1981; SMITH, 1978; WYNN-WILLIAMS, 1980).

Following laboratory simulations of the effects of physical and edaphic factors on total peat respiration and microbial biomass (WYNN-WILLIAMS, 1982), ancillary experiments were conducted to determine if predation by nematodes and Protozoa was contributory to the post-spring microbial decline.

II. Methods

Full details of sampling techniques, microbial enumeration and Gilson respirometry are given in WYNN-WILLIAMS (1979) and laboratory simulations in WYNN-WILLIAMS (1982).

Intact peat core sections from the 1-3 cm depth zone of a *Polytrichum alpestre* turf were injected aseptically into iso-diametric (27 mm) base cones of special Gilson respirometer flasks for treatment, incubation and respirometry at ambient field temperature.

Viable bacteria were counted on spread plates of casein peptone starch agar enriched with peat expressate and containing Actidione (Upjohn) at 50 mg l^{-1}. Viable yeasts and fungi were counted on Sabouraud dextrose agar containing Aureomycin (Lederle) at 30 mg l^{-1} and sodium propionate (250 mg l^{-1}) to control fungal overgrowth. Counts were converted into biomass assuming the following : the mass of an average bacterium is 1.5 pg (ALEXANDER, 1961); that of a yeast is 41.47 pg (SAITO, 1955); the S.G. of protoplasm is 1.1 (SAITO, 1955); the diameter of a tundra

fungal hypha is 2 μm (BÅÅTH & SÖDERSTRÖM, 1979); the length of a fungal colony-forming unit (CFU) in *Polytrichum* peat is 6.0 μm (WYNN-WILLIAMS, 1982), consequently the mass of a fungal CFU is 21.3 pg. For microbial enumeration, replicate cores were incubated under conditions identical to those in the respirometer but were sub-sampled to obtain *ca* 1 g of peat down the profile, before and after incubation. The resulting holes were plugged to maintain conditions of aeration and drainage similar to cores in the respirometer.

Natural populations of live nematodes were extracted from 0-3 cm layer of *Polytrichum* at the SIRS by a modified Baermann funnel technique. After concentration by settling and determination of the composition and density of the populations using a Doncaster dish and x25 magnification (CALDWELL, 1981), 0.5 ml aliquots were pipetted evenly onto the surface of four replicate peat cores for respirometry relative to four water-amended control cores.

A natural population of Protozoa was extracted from the 5-7 cm layer of *Polytrichum* turf at the SIRS by cultivation on streak plates of *Klebsiella aerogenes*. The mixed culture of flagellates and testate amoebae was subsequently grown in Erdschreiber medium on agar plates (SMITH, 1978). These were supplemented with cultures of the testate amoebae *Euglypha rotunda* and *Trinema lineare* (courtesy of Dr. C.G. OGDEN), both of which occur in the maritime Antarctic, to ensure a mixture of potential fungivores and bacterivores. Protozoa were extracted by enclosing the intact experimental cores in nylon mesh bags within polythene sheaths and compressing them between rollers. The expressate was concentrated by centrifugation and counted under phase-contrast in a 1 ml counting chamber. Two millilitres of the concentrated protozoan suspension were pipetted evenly onto five replicate cores in special Gilson flasks. Six replicate cores were amended with sterile distilled water only. Four of each set were subsampled for microflora before and after prolonged incubation.

Three simulations were carried out with *Polytrichum* cores. Firstly, for 216 h at 5°C with a mainly bacteria-feeding nematode population added to cores taken frozen in October 1975. Secondly, for 453 h at 1.5°C with a mainly fungal-feeding nematode population added to cores taken frozen in October 1976. Thirdly, for 309 h at 1.5° C with the mixed flagellate and testate amoeba population added to cores taken during the autumn freeze-thaw period of March 1979 (returned to England at -10° C).

III. Results

During the 1975-76 spring, the sharp decline in total peat O_2-uptake after the spring peak was paralleled by a similar decline in bacteria and yeasts. The decline in fungal CFU was delayed. When combined, these staggered responses made the total change in microbial biomass insignificant. However in spring 1976-77, the decline

in aerobic respiration after the initial peak was paralleled by all three microbial groups which could therefore be combined (Fig. 1). No two years are climatically the same. However, studies at Signy Island of testate amoebae in *Polytrichum* in 1970-71 (SMITH, 1981) and nematodes in *Polytrichum* at the SIRS in 1977-78 (CALDWELL, 1981) showed that these microfaunal populations increased as or after the microbial biomass decreased (Fig. 1). These observations provoked the present laboratory simulations.
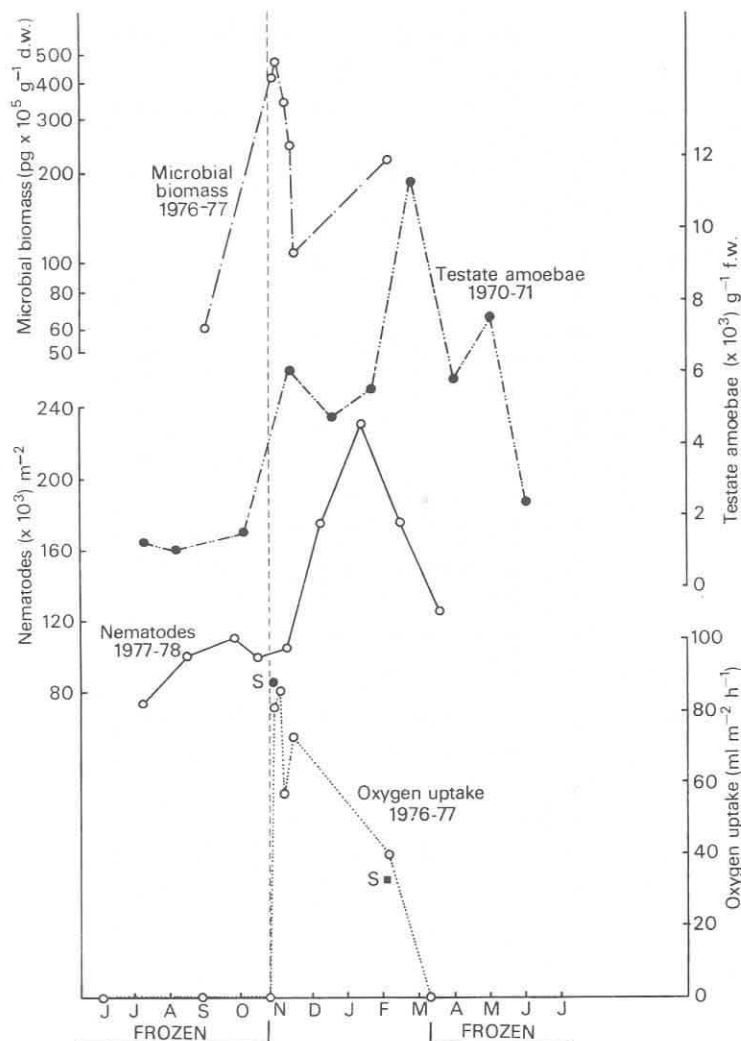

Figure 1 The response of total peat respiration, total microbial biomass and counts of nematodes and testate amoebae to the spring thaw in the top 3 cm of *Polytrichum* turf at Signy Island. S ● and S ■ indicate laboratory simulations of spring 1976 and autumn 1977 respectively. The sampling data for nematodes and amoebae have been adjusted so that the date of the spring thaw coincides in all the years given.

240

TABLE I

Microbial predation by two natural nematode populations added to intact *Polytrichum* peat cores.

Microbial group	Treatment	60 %-bacterivorous nematode population			69 %-fungivorous nematode population		
		% Change in microbial biomass	Microbial biomass consumed (pg) nematode^{-1} min^{-1} at 5° C	% Change in total O$_2$-uptake (± SEM)	% Change in microbial biomass	Microbial biomass consumed (pg) nematode^{-1} min^{-1} at 1.5° C	% Change in total O$_2$-uptake (± SEM)
Bacteria	Water	+ 1 040	negligible	-	+ 945	negligible	-
	Nematodes	− 13	360	-	+ 512	20	-
Yeasts	Water	+ 660	negligible	-	+ 870	negligible	-
	Nematodes	− 87	90	-	+ 400	365	-
Total	Water	+ 720	negligible	+ 29.7 (± 8.3)	+ 877	negligible	variable
	Nematodes	− 150	450	− 16.3 (± 11.7)	+ 403	385	variable

Table I shows that in the absence of nematodes, both the yeast and bacterial biomass increased greatly. In both simulations the initial bacterial biomass of the water-amended cores was similar (1 540 and 1 780 pg.g^{-1} d. wt. respectively) and their percentage increase was also similar despite the different incubation conditions. The yeast biomass was half as large in the spring 1975 simulation as in that of 1976 (7 760 and 15 760 pg.g^{-1} d. wt. respectively). Corrections for microbial growth rate were applied when calculating predation rates.

The bacterivore-dominated population consisted mainly of *Teratocephalus* (54 %) and *Plectus* (5 %) relative to *Aphelenchoides* (26 %) and *Ditylenchus* (9 %). The fungivore-dominated population contained *Aphelenchoides* (63 %) and *Ditylenchus* (9 %) relative to *Teratocephalus* (15 %) and *Plectus* (10 %). The consumption of bacterial cells by nematodes differed between the bacterivore and fungivore simulations (238 and 13 bacteria per nematode per min at 5° and 1.5° C respectively). This was also true for the yeasts (2.3 and 8.8 yeasts per nematode per min respectively). This effect was not entirely temperature-dependent as shown by the lower yeast consumption at the higher temperature.

In the first experiment there was also a net decrease in O_2-uptake relative to an increase in water-amended controls. Variation between replicate cores made respiratory data inconclusive in the second simulation.

The composition of the 2-ml protozoan suspension used in the third simulation was as follows : potentially yeast-feeding testate amoeba *Euglypha rotunda* (42 000) and *Trinema lineare* (18 000), and potentially bacteria-feeding flagellates *Bodo saltans* and *Oikomonas termo* (combined total 1 240 000). In the absence of Protozoa, yeast growth was nearly exponential (+ 97 600 %) but was limited to + 5 340 % in their presence. Bacteria increased by + 6 250 % in the absence of Protozoa, but decreased − 6 % in their presence. The combined effect of the Protozoa was to limit a + 64 740 % increase in microbial biomass to only + 50 %. Nevertheless, the decrease in O_2-uptake during these changes was the same (− 44 %) whether in the presence or absence of added Protozoa.

During the incubation, the population of testae amoebae declined by - 70 % before its increase could be monitored. However the flagellate population increased by + 130 % in the same period, although this might have been similarly sub-maximal. Assuming that only the flagellates consumed bacteria in the present study, the predation-rate was 23 bacteria per flagellate per day at 1.5° C as calculated by HEAL (1967). This method could not be used for the testate amoebae because of the demise of the population. However, assuming that the population of testate amoebae remained constant and consumed mainly yeasts, consumption was *ca* 4.3 yeasts per testate per day.

IV. Discussion

In both years, the yeast population declined rapidly after the spring bloom. Under certain field conditions all the heterotrophs, bacteria, yeasts and fungi, may respond similarly, such as after the spring of 1976. The similarity between field-fresh spring sample respiration and laboratory-simulated spring respiration in 1976 (Fig. 1) justified extrapolation of nematode-predation simulation data to the field situation. This was also true for autumn-sampled peat, supporting the suitability of the material used for the protozoan predation study.

The initial part of the laboratory simulation of spring 1976 showed exponential growth of yeasts during the freeze-thaw cycles (WYNN-WILLIAMS, 1980, 1982). The bacteria did not grow as rapidly under these conditions, possibly because of competition for DOC (dissolved organic carbon) by the yeasts. Sustained exponential growth in the absence of continuing freeze-thaw cycles was unlikely so that the calculated predation-rate was exaggerated. In view of the high density of nematodes added, it was assumed that the population would remain relatively constant due to competition for food. Continual grazing may stimulate rapid microbial production by restricting competition.

Comparison of the consumption rates of the two nematode populations showed that the greater the numerical dominance of a feeding-group, the greater its individual microbial predation. Dominance may therefore depend on rapid consumption. This rate was also dependent on the characteristics of the dominant species. A large nematode such as *Plectus* will consume bacteria at a faster rate than smaller worms.

For analytical purposes, it was assumed that predation by the very small indigenous nematode population in the samples was negligible relative to the additions. DAVIS (1981) concluded that in *Polytrichum* communities at the SIRS the microflora made up 88.1 % of the nematode diet. However, the nematode biomass in this peat community was only 1.6 % of the total faunal biomass whereas the Protozoa constituted 78.5 %. Hence microbial predation by nematodes relative to Protozoa was of limited significance at natural population densities. This was also concluded by SØHLENIUS (1979) who estimated that in Swedish coniferous soil, predation by nematodes accounted for only 2 % of the bacterial production. DAVIS (1981) considered that the diet of flagellates (Mastigophora) at the SIRS was uncertain but may comprise 95 % microflora and 5 % dead organic matter (DOM). Alternatively, some saprozoic flagellates may rely entirely on DOM. There is also uncertainty about the testate amoebae (Sarcodina) whose diet could range from 95 % heterotrophic microflora and 5 % algae to 50 % of each. Ciliates were of limited significance at the SIRS (SMITH, 1978).

The present study indicated that the microflora is a major food source for both groups of Protozoa. Assuming that the viable counts of yeasts and the direct counts of amoebae in the present experiment represented the viable populations present in the

peat, the initial ratio of yeasts to amoebae was 1 : 1. Studies of feeding-rates of *Acanthamoeba* on yeasts (HEAL, 1967) showed that at such a ratio the feeding-rate would be 4-19 yeasts per amoeba per day which was similar to the present testates (*ca* 4.3 yeasts per amoeba per day) despite their cell volume being 2-3 x greater. The viable count method of enumerating yeasts in the present study under-estimates the population so this rate will be a low estimate. The present feeding-rate was equivalent to an *Acanthamoeba* generation-time of 0.1-0.6 generations per day which would require an incubation temperature of up to 25° C. Similar rates for Antarctic strains at 1.5° C suggest a psychrotolerant enzyme-system. However, the amoebic population crash made these conclusions tentative.

In Swedish coniferous forest humus, CLARHOLM (1981) showed that naked amoebae, absent from the SIRS, were the most significant predators of bacteria. However, in the Swedish study no reference was made to yeasts which were highly significant in the SIRS ecosystem (WYNN-WILLIAMS, 1980; 1982). The predation-rate derived from CLARHOLMS's (1981) data was *ca* 490 bacteria per amoeba per day. In biomass terms, this was equivalent to 18 yeasts per amoeba per day, rather more than the present estimate but still similar to it and to the rate for *Acanthamoeba*. Despite the increase in flagellates observed here, both DAVIS (1981) and CLARHOLM (1981) concluded that the amoebae were the only group of Protozoa of sufficient total biomass *in vivo* to affect the microflora significantly, consistent with the sequential correlation shown in Fig. 1. The discrepancy between the present consumption rate for flagellates (*ca* 1 bacterium per hour at 1.5° C) including *Bodo*, mand *Pleuromonas jaculans* (1300 per hour at 20° C) (FENCHEL, 1982) which is morphologically similar, may be partly empirical and partly due to saprozoism.

Apparent consumption rates by Protozoa may be exaggerated by concurrent microbial predation by nematodes. Conversely, the nematodes may predate the smaller Protozoa. However, nematode activity was found to be insignificant in the unamended cores of the simulations. In these simulations, several other assumptions were made. 1. All the added microfauna is viable and feeds at a constant rate according to predicted diets without interactions; 2. Feeding on fungal hyphae, and on algae at depth 1-3 cm in the dark, is negligible; 3. Temperature differences do not have a major influence on feeding and growth rates in this Antarctic ecosystem (WYNN-WILLIAMS, 1982).

The decline in O_2-uptake in the presence of nematodes relative to water controls in the first simulation suggested that a decrease in biomass was responsible. This was supported by the less distinct biomass changes in the second simulation being associated with excessively variable O_2-uptake. However, the absence of any change in respiration rate with or without Protozoa, despite the large changes in biomass, showed that biomass and activity are not necessarily related.

It is concluded from field and laboratory simulation studies that Protozoa, and to a lesser extent nematodes, contribute to the post-spring decline in microbial biomass and activity in *Polytrichum* peat at the SIRS.

Acknowledgements

References

ALEXANDER (M.), 1961. — Introduction to Soil Microbiology. — *Wiley, New York*, 220 p.

BÅÅTH (E.) & SÖDERSTRÖM (B.E.), 1979. — The significance of hyphal diameter in calculation of fungal biovolume. — *Oikos*, **33** : 11- 14.

CALDWELL (J.R.), 1981. — Biomass and respiration of nematode populations in two moss communities at Signy Island, maritime Antarctic. — *Oikos*, **37** : 160-166.

CLARHOLM (M.), 1981. — Protozoan grazing of bacteria in soil – Impact and importance. — *Microbial Ecology*, **7** : 343-350.

COLLINS (N.J.), BAKER (J.H.) & TILBROOK (P.J.), 1975. — Signy Island, Maritime Antarctic. — *In* T. ROSSWALL & O.W. HEAL (Eds.), *Structure and Function of Tundra Ecosystems — Ecological Bull.* (Stockholm), **20** : 345-374.

DAVIS (R.C.), 1981. — Structure and function of two Antarctic terrestrial moss communities. — *Ecological Monographs*, **5** :125-143.

FENCHEL (T.), 1982. — Ecology of heterotrophic microflagellates. II. Bioenergetics and growth. — *Marine Ecology — Progress Series*, **8** : 125-143.

HEAL (O.W.), 1967. — Quantitative feeding studies of soil amoebae. — *In* O. GRAFF & J.E. SATCHELL (Eds.), *Progress in Soil Biology.* — North Holland Publishing Company, Amsterdam : 120-126.

SAITO (T.), 1955. — The significance of plate counts of soil fungi and the detection of their mycelia. — *Ecological Reviews*, **14** :64-74.

SMITH (H.G.), 1978. — Distribution and ecology of terrestrial Protozoa of sub-Antarctic and maritime Antarctic islands. — *British Antarctic Survey Scientific Report*, **95** : 104 pp.

SMITH (H.G.), 1981. — A comparative study of the terrestrial Protozoa inhabiting moss turf peat on Iles Crozet, South Georgia and the South Orkney Islands. — *Colloque sur les Ecosystèmes Subantarctiques*, CNFRA No. 51, Paimpont : 137-145.

SØHLENIUS (B.), 1979. — A carbon budget for nematodes, rotifers and tardigrades in a Swedish coniferous forest soil. — *Holarctic Ecology*, **2** : 30-40.

WYNN-WILLIAMS (D.D.), 1979. — Techniques used for studying terrestrial microbial ecology in the maritime Antarctic. — *In* A.D. RUSSEL & R. FULLER (Eds.), *Cold Tolerant Microbes in Spoilage and the Environment.* — Society for Applied Bacteriology Technical Series, **13**, Academic Press, London : 67-81.

WYNN-WILLIAMS (D.D.), 1980. — Seasonal fluctuations in microbial activity in Antarctic moss peat. — *Biological Journal of the Linnean Society*, **14** : 11-28.

WYNN-WILLIAMS (D.D.), 1982. — Simulation of changes in microbial activity of maritime Antarctic peat. — *Soil Biology and Biochemistry*, **14** : 1-12.

Discussion

HASSALL, M : To what extent is the burst of microbial activity and its subsequent decline due to an increase in available substrate following the thaw ?

WYNN-WILLIAMS, D.D. : The majority of this activity is due to the release of dissolved organic carbon from frost-damaged cells, and the depletion of this material by microorganisms is probably mainly responsible for the decline in respiratory activity. Predation of microbial biomass is only an additional contributory factor to the decline and is more of a fine adjustment to the system.

★
★ ★